Results in Physics 18 (2020) 103277

Contents lists available at ScienceDirect

Results in Physics

journal homepage: www.elsevier.com/locate/rinp

Mechanism of interaction among nanocrystalline carbonate-substituted hydroxyapatite and polar amino-acids for the biomimetic composite technology: Spectroscopic and structural study

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ARTICLE INFO

Keywords:
Biomimetic composite
Polar amino acids
Nanocrystalline carbonate-substituted hydroxyapatite
Mechanism of the interaction
Foreign ion
Molecular vibrational spectroscopy

ABSTRACT

This study investigated changes in the interaction of B-type non-stoichiometric nanocrystalline carbonate-substituted hydroxyapatite (nano-CHAP) with polar amino acids (AA) with respect to CHAP-AA linking. Molecular vibrational spectroscopy revealed for the first time that the surface interaction of polar amino acids with defective nano-CHAP is determined not only by the charge state of AA but can be mediated by a foreign ion conjugated with the lateral bonds of amino acids. Understanding this mechanism of CHAP-AA interaction is required for the development of the new generation of dental biomimetic materials based on HAP as well as for their qualitative integration with the amino acid matrix of the dental tissues.

Introduction

The production of natural-like organic-mineral composites with controllable morphology at the micro- and nano-levels, controlled physicochemical properties and perfect interaction between components is challenging [1–3]. For dentistry purposes, synthesis of such biomimetic materials using nanocrystalline hydroxyapatite (HAP) and amino acids (AA) makes it possible to reproduce the composition of the human dental tissue, to link HAP in the composite matrix and configure its morphological organisation [4–6]. However, the biogenic apatite of dental tissue is imperfect in terms of its crystal structure [7], thus, HAP-AA interactions remain an issue in the incorporation of the foreign ions into the crystalline lattice of apatite which can interact with the lateral bonds of amino acids [8]. Recent investigations have shown that the crucial factor in the formation of biocomposites highly active relative to the dental tissues and the osseous system is the interaction between nano-CHAP and AA [1,2,6]. In [6,9,10], it was determined that the interaction of nano-CHAP-AA can take place through 1) the formation of a bond between phosphorus-oxygen tetrahedrons of HAP and positively charged amide groups of amino acids, 2) the substitution of an OH group in nano-CHAP with the carboxylic group of AA or its bond with positively charged calcium ions, 3) the incorporation of COO groups into the structure of nano-CHAP to the defect positions. The type of interaction depends on the amino acid, its isoelectric point and the pH of the medium as well as the structure and defects in the nano-CHAP lattice [2,6,9,11]. Also, it is known that the bond of AA-nano-CHAP can be mediated by the foreign ions thus changing the type of interaction in the conditions of a single charge state of the amino acid [10]. Taking into account the saturation of native dental tissue and HAP based biomimetic composite with different ions [7,12] and the defective structure of nano-CHAP [2,8,13], the issues regarding the interaction between the amino acid complex and nano-CHAP in the formation of natural-like composites remain unclear, therefore require a thorough investigation. For this reason, this study aimed to investigate the possible mechanisms of CHAP-AA interaction in the dental biomimetic composite by optical molecular microspectroscopy.

Methods

Biomimetic composites modelling the organic and mineral composition of native enamel and dentin were synthesised using inorganic and organic components. The inorganic component of the elaborated biocomposite was nanocrystalline carbonate-substituted calcium hydroxyapatite (CHAP). B-type nano-CHAP was obtained from a biogenic source of calcium (eggshell) and a water suspension of nano-CHAP was prepared by the precipitation method, by titrating annealed eggshell powder (calcium hydroxide, $Ca(OH)_2$) with 0.3 M orthophosphoric acid H_3PO_4 as described previously [14]. The organic component of the

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https://doi.org/10.1016/j.rinp.2020.103277
Received 8 May 2020; Received in revised form 6 July 2020; Accepted 15 July 2020
Available online 31 July 2020

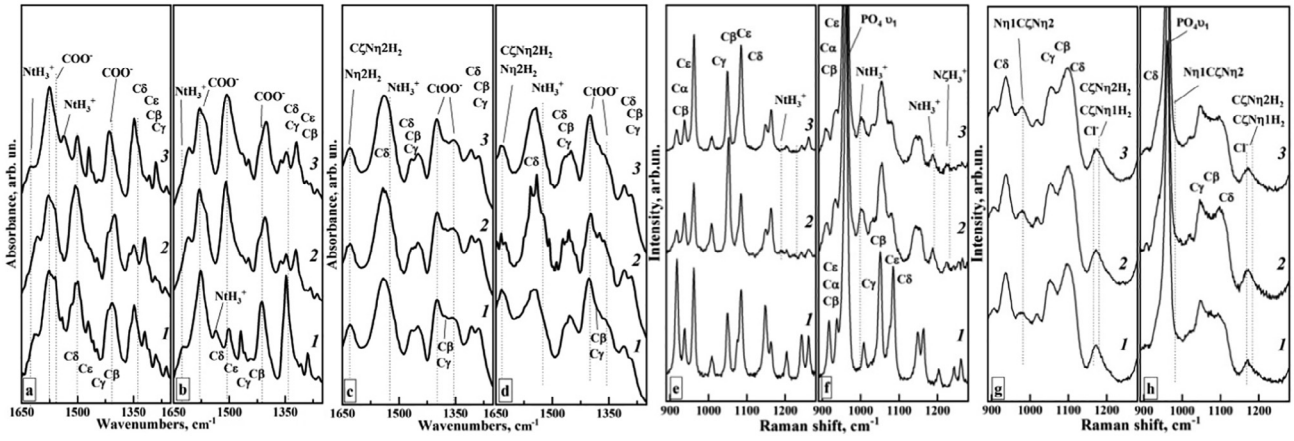

Fig. 1. IR absorbance (a, b, c, d) and Raman spectra (e, f, g, h) of biomimetic organic-mineral composites in the media with different pH: 1 – pH ≤ 5; 2 – pH ≥ 7.55; 3 – pH ≥ 11.2. (a, e) L-LysHCL in pure form; (b, f) L-LysHCL + nano-CHAP; (c, g) L-ArgHCL in pure form; (d, h) L-ArgHCL + nano-CHAP.

synthesised biomimetic composite was a mix of basic polar amino acids found in the enamel dental tubule. The nano-CHAP suspension was subjected to ultrasound treatment (55 W) for 5 min before introduction into amino acid solutions. To register the change in the charge state of amino acids, aqueous solutions of L-lysine hydrochloride ($C_6H_{15}ClN_2O_2$) and L-arginine hydrochloride ($C_6H_{15}ClN_4O_2$) (n = 10 mg/ml) were prepared with different pH values (≥11.2, ≥7.55 and ≤5), then aqueous solutions of NH_4OH and HCl were added. The interaction of AA-nano-CHAP in the biomimetic composite was studied by FTIR spectroscopy (Vertex-70 PlatimunATR, Bruker) and Raman microspectroscopy (RamMix 532, EnSpectr, λ = 532 nm). Infrared absorbance spectra were recorded with the resolution of 4 cm^{-1} within the range of 400–4000 cm^{-1} using 16 co-added scans. Raman spectra were recorded with the resolution of 2 cm^{-1} in the range of 200–4000 cm^{-1} using 30 co-added scans.

Results

The vibrational spectra of the pure amino acid complexes (Fig. 1 a, c, e, g) and those in which nano-CHAP was introduced into AA solutions (b, d, f, h) are presented in Fig. 1. The change in vibrational characteristics of the lateral bonds in amino acids in the IR (Fig. 1, a, b, c, d) and Raman spectra (Fig. 1 e, f, g, h) represent the local change in the conformational environment of AA under the formation of the bond with the defective structure of nano-CHAP dependent on pH (pH ≥ 11.2, ≥7.55 and ≤5). The samples of L-lysine (a, b, e, f) shows mutual changes in intensity for the vibrational modes of COO^- and NH_3^+ groups in different pH and after the introduction of nano-CHAP into the AA solution, thus confirming the change in the charge state in L-lysine with respect to [10]. It is of note that in the presence of nano-CHAP in the medium with a pH < 5, the intensity of vibrations of COO and CH-groups in the main chain of the AA molecule increased (Fig. 1 b), while for pH ≥11.2 the increase of vibrational bands observed in the range of 1187 and 1225 cm^{-1} is related to NH_3^+ [10,15]. This AA behaviour differs from that proposed in [9,10] and can be explained by the defective nature of nano-CHAP shown in [14] and by the presence of vacancies in the OH and PO_4^{3-} positions, as well as by the charge compensation during the interaction of nano-CHAP with AA.

Comparison of the spectra of L-arginine samples (c, d, g, h) obtained from solutions with the addition of nano-CHAP at pH ≥ 11.2, ≥7.55 and ≤5 show a small change in the intensity of C-N1-H_2 mode for [-NH-C-$(NH_2)_2$]$^+$ guanidine group, localised at 1630 cm^{-1} in the IR spectrum [15] and 980 cm^{-1} in the Raman spectrum [10]. In addition, there is also a change in the intensity of the NH_3^+ mode (1150–1250 cm^{-1}) in the Raman spectra [16,17]. Low activity of these molecular groups during the formation of L-ArgHCL-nano-CHAP bond is explained by the binding of a guanidine group with a chlorine atom, mediated by desorption of this ion via the AA molecule [9,10]. It should be noted that for all the biocomposite samples, the introduction of nano-CHAP into the amino acid solutions (b, d, f, h) affects the change in intensity of the vibrations for carbon atoms in the main chain Cα, Cβ, Cγ, Cδ, Cε [10], corresponding to the different orientation of AA molecules in the presence of nano-CHAP.

Conclusions

Raman and FTIR spectroscopy of biomimetic composites synthesised from AA complexes and nano-CHAP crystals in alkali, neutral and acidic media revealed changes in the AA-nano-CHAP interactions due to the transformation of the charge state of the amino acid. The spectroscopic data regarding the formation of the biomimetic composite showed that the formation of the AA-nano-CHAP bond depends on the type of defect in the crystalline lattice of nano-CHAP and the presence of foreign ions in the biocomposite.

Funding

The study was performed under support of Russian Foundation for Basic Research (RFBR) grant №18-29-11008 mk and grant of the President of Russian Federation №МК-419.2019.2.

CRediT authorship contribution statement

D.L. Goloshchapov: Conceptualization, Methodology, Investigation, Writing - original draft. **Yu.A. Ippolitov:** Investigation. **P.V. Seredin:** Conceptualization, Methodology, Funding acquisition, Supervision, Investigation, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Lagazzo A, Barberis F, Carbone C, Ramis G, Finocchio E. Molecular level interactions in brushite-aminoacids composites. Mater Sci Eng C Mater Biol Appl 2017;70:721–7. https://doi.org/10.1016/j.msec.2016.09.030.

[2] Seredin PV, Goloshchapov DL, Prutskij T, Ippolitov YA. Fabrication and characterisation of composites materials similar optically and in composition to native dental tissues. Results Phys 2017;7:1086–94.. https://doi.org/10.1016/j.rinp.2017.02.025.

[3] Antoniac IV, editor. Handbook of Bioceramics and Biocomposites Cham: Springer

International Publishing; 2016. https://doi.org/10.1007/978-3-319-12460-5.
[4] Palazzo B, Walsh D, Iafisco M, Foresti E, Bertinetti L, Martra G, et al. Amino acid synergetic effect on structure, morphology and surface properties of biomimetic apatite nanocrystals. Acta Biomater 2009;5:1241–52. https://doi.org/10.1016/j.actbio.2008.10.024.
[5] Yao Y, Shao H, Zhang Q. Development and Characterization of a Novel Amorphous Calcium Phosphate/Multi (Amino Acid) Copolymer Composite for Bone Repair. J Biomater Tissue Eng 2015;5:387–90. https://doi.org/10.1166/jbt.2015.1321.
[6] Tavafoghi M, Cerruti M. The role of amino acids in hydroxyapatite mineralization. J R Soc Interface 2016;13:20160462. https://doi.org/10.1098/rsif.2016.0462.
[7] Teaford MF, Smith MM, Ferguson MWJ. Development. Function and Evolution of Teeth: Cambridge University Press; 2007.
[8] Cacciotti I. Multisubstituted hydroxyapatite powders and coatings: The influence of the codoping on the hydroxyapatite performances. Int J Appl Ceram Technol 2019;16:1864–84. https://doi.org/10.1111/ijac.13229.
[9] Comeau P, Willett T. Impact of Side Chain Polarity on Non-Stoichiometric Nano-Hydroxyapatite Surface Functionalization with Amino Acids. Sci Rep 2018:8. https://doi.org/10.1038/s41598-018-31058-5.
[10] Hernández B, Pflüger F, Derbel N, De Coninck J, Ghomi M. Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VI. Amino Acids with Positively Charged Side Chains: L-Lysine and L-Arginine. 114:1077–88 J Phys Chem B2010. https://doi.org/10.1021/jp909517r.
[11] Cacciotti I. Cationic and Anionic Substitutions in Hydroxyapatite. In: Antoniac IV, editor. Handb. Bioceram. Biocomposites, Cham: Springer International Publishing; 2016, p. 145–211. https://doi.org/10.1007/978-3-319-12460-5_7.
[12] Buddhachat K, Klinhom S, Siengdee P, Brown JL, Nomsiri R, Kaewmong P, et al. Elemental Analysis of Bone, Teeth, Horn and Antler in Different Animal Species Using Non-Invasive Handheld X-Ray Fluorescence. PLoS ONE 2016;11:e0155458https://doi.org/10.1371/journal.pone.0155458.
[13] Domashevskaya EP, Al-Zubadi AA, Goloshchapov DL, Rumyantseva NA, Seredin PV. Structure and composition of metal-substituted calcium-deficient hydroxyapatite. J Surf Investig X-Ray Synchrotron Neutron Tech 2014;8:1128–36. https://doi.org/10.1134/S1027451014060032.
[14] Goloshchapov DL, Lenshin AS, Savchenko DV, Seredin PV. Importance of defect nanocrystalline calcium hydroxyapatite characteristics for developing the dental biomimetic composites. Results Phys 2019;13:102158https://doi.org/10.1016/j.rinp.2019.102158.
[15] Barth A. The infrared absorption of amino acid side chains. Prog Biophys Mol Biol 2000;74:141–73. https://doi.org/10.1016/S0079-6107(00)00021-3.
[16] Freire PTC, Barboza FM, Lima JA, Melo FEA, Filho JM. Raman Spectroscopy of Amino Acid Crystals. In: Maaz K, editor. Raman Spectrosc. Appl., InTech; 2017. https://doi.org/10.5772/65480.
[17] Aliaga AE, Garrido C, Leyton P, Diaz FG, Gomez-Jeria JS, Aguayo T, et al. SERS and theoretical studies of arginine. Spectrochim Acta A Mol Biomol Spectrosc 2010;76:458–63. https://doi.org/10.1016/j.saa.2010.01.007.